

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT (PCT Article 36 and Rule 70)

REC'D 23 AUG 2005

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Applicant's or agent's file reference Cal 86465		<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/EP 03/06839	International filing date (day/month/year) 26.06.2003	Priority date (day/month/year) 26.06.2003	
International Patent Classification (IPC) or both national classification and IPC C12N15/10			
Applicant METAPONTUM AGROBIOS S.R.L.			

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
  - ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:
  - I ☒ Basis of the opinion
  - II ☐ Priority
  - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
  - IV ☐ Lack of unity of invention
  - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - VI ☐ Certain documents cited
  - VII ☐ Certain defects in the international application
  - VIII ☐ Certain observations on the international application

Date of submission of the demand  24.01.2005	Date of completion of this report  24.08.2005
Name and mailing address of the international preliminary examining authority:   European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer  Oderwald, H  Telephone No. +31 70 340-4274  

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/EP 03/06839

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-26 as originally filed

**Claims, Numbers**

1-6 received on 15.06.2005 with letter of 10.06.2005

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes: Claims	1-6
	No: Claims	
Inventive step (IS)	Yes: Claims	1-6
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-6
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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**Re Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following documents:

- D1: SANO H ET AL: 'A NOVEL RAS-RELATED RGP1 GENE ENCODING A GTP-BINDING PROTEIN HAS REDUCED EXPRESSION IN 5 AZACYTIDINE-INDUCED DWARF RICE' MOLECULAR & GENERAL GENETICS, vol. 228, no. 1-2, 1991, pages 227-232, XP002213260 ISSN: 0026-8925
- D2: SANO H ET AL: 'A SINGLE TREATMENT OF RICE SEEDLINGS WITH 5 AZACYTIDINE INDUCES HERITABLE DWARFISM AND UNDERMETHYLATION OF GENOMIC DNA' MOLECULAR & GENERAL GENETICS, vol. 220, no. 3, 1990, pages 441-447, XP002213261 ISSN: 0026-8925
- D3: WO 96/36693 A (PHYTERA INC) 21 November 1996 (1996-11-21)

**NOVELTY (Art. 33(2) PCT) AND INVENTIVE STEP (Art. 33(3) PCT)**

The document D1 is regarded as being the closest prior art to the subject-matter of claims 1, and shows (the references in parentheses applying to this document):

A method for the isolation of expressed sequence tags (ESTs) comprising the step of: (a) exposure (soaking) of rice seedlings to 5-aza-cytidine in quantities ranging from 0,1 mM to 2 mM (0,3 mM, see D2), (b) extraction of the nucleic acids (poly(A)<sup>+</sup> RNA) from the shoots (seedlings), (c) synthesis of a cDNA library and (d) sequencing and sequence analysis of the clones of the library. The methods has been carried out at a temperature of 26°C (see D2).

The subject-matter of claims 1-6 is therefore **new** (Article 33(2) PCT).

The subject-matter of claim 1 differs from this known method in that in step (a) seeds are germinated in the presence of 5-aza-cytidine. This results in seedlings with activation of genes expressed in different developmental phases of the plant ('totipotent' seedlings).

**INTERNATIONAL PRELIMINARY  
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The problem to be solved by the present invention may be regarded as providing an method for the isolation of ESTs from different developmental phases of plants.

The solution is a method in which seeds are germinated in the presence of 5-aza-cytidine.

The germinating of seeds soaked in 5-aza-cytidine has been described in document D3. The document, however, discloses a method of affecting secondary metabolite production in a plant.

The cited prior art documents -either taken alone or in combination- neither disclose nor suggest a method for isolating ESTs using seeds germinated in the presence of 5-aza-cytidine.

The same applies *mutatis mutandis* to claims 2-6.

The present application therefore meets the criteria of Article 33(1) PCT, because the subject-matter of claims 1-6 involves an inventive step in the sense of Article 33(3) PCT.

EPO - DG 1

15. 06. 2005

(57)

CLAIMS

1. A method for the isolation of expressed sequence tags in different development phases of plants which comprises the following steps:
- 5 (a) germination of the seeds in a suitable medium in the presence of 5-azaCytidine in quantities ranging from 0.1 mM to 2 mM;
- (b) extraction of the nucleic acids from the shoots grown as specified in step (a);
- 10 (c) synthesis of the cDNA library starting from the nucleic acids extracted in step (b);
- (d) sequencing and sequence analysis of the clones of the library. [NEW PAGE 27A]
- [2]
- 3 2. The method according to claim 1, wherein the quantity of azaCytidine in step (a) ranges from 0.3 mM to 0.5 mM.
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- 4 3. The method according to claim 1, wherein in step (a) the germination is carried out at a temperature ranging from 20 to 30°C.
- 20 4. The method according to claim 3, wherein the temperature ranges from 22 to 26°C.
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- 6 5. A kit for the synthesis of cDNA libraries of plants comprising the necessary components and instructions for the embodiment of the method defined in claim 1.
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2. The method according to claim 1 wherein said step  
(a) of germination of seeds has a duration of 21 days.

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